REMARKS

Reconsideration is requested.

Claims 1-87, 91-100, 103 and 106 have been canceled, without prejudice. Claims 110 and 111 have been added. No new matter has been added. Claims 88-90, 101-102, 104-105 and 107-111 are pending.

Support for the amendments to the claims may be found, for example, in the specification in the following passages:

page 2, lines 9-17, and 18 onwards over to page 19;

page 18, lines 5-21;

page 21, line 24 to page 22, line 22;

page 23, line 3;

page 31, line 24 to page 32, line 11;

page 38, lines 12-16;

page 40, line 26 to page 42, line 6;

page 47, line 25 to page 48, line 8;

page 55, line 26 to page 56, line 11;

Example 29, 32, 36...; and

page 74, lines 10-25.

Claim 107 has been amended to be dependent on claim 88 and claim 109 has been made dependent on claim 107. New claims 110 and 111 are similar to existing claims 105 and 109. No new matter has been added.

The present claims are submitted to define a single invention which reflect the applicants discovery of positional and abundance alterations in expression of MCM protein at the surface of tissue in dysplastic or neoplastic cellular growth abnormality. The claimed invention, as further discussed below, provides methods for sampling and testing of cells taken or released from epithelial tissue surfaces in clinical diagnosis and is neither taught nor suggested by the art of record.

The Examiner's comments with regard to priority are noted and no further comment is believed to be required. The claimed invention is patentable over the art of record for the reasons described in the following detailed remarks.

The Section 102 rejections of claims 88-100, 105, Werness et al., (Laboratory Investigation, Vol. 76, No. 1, page 185A, March 1997) and Todorov et al. (Laboratory Investigation, January 1887, Vol. 78, No. 1, pages 73-78), both of which refer to a protein BM28, are traversed. Reconsideration and withdrawal of the Section 102 rejection are requested in view of the following distinguishing remarks. The applicants acknowledge that BM28 is MCM2.

The pending claims provide a method wherein the test sample contains cells and "comprises a specimen selected from the group consisting of sputum, bronchio-alveolar lavage specimens, urine, breast duct fluid, brushings from the alimentary tract, and cervical, fecal or urine cytology smears". This subject-matter reflects the inventors' novel and non-obvious finding of abnormal expression of the target polypeptide at the epithelial surface of tissues when there is dysplasia or neoplasia. The abnormal expression at the surface of epithelial surfaces allows for detection in

brushings/scrapings of the surface and in fluid into which cells have sloughed or exfoliated.

From their work, the present inventors discovered that MCMs are present at the appropriate abundance and in the appropriate micro-anatomical locations to enable them to be used for detection of neoplastic conditions (both malignant and pre-malignant) by analysis of cells taken or released from the surface of tissue. Such cells can be sampled either actively (e.g. in a smear, brushing or washing) or passively (e.g. via exfoliation into a fluid such as urine or onto the surface of stool).

The inventors further observed that MCMs show superior sensitivity compared to PCNA and Ki67 for detection of neoplastic cells at all anatomical sites examined. This was not identified by the cited prior art, including Werness/Todorov *et al.*

They observed staining of the majority of cells at the surface of malignant neoplasms. This was not indicated by Werness/Todorov *et al.*

On examination of pre-malignant conditions, the present inventors showed very frequent expression of MCMs, including in surface cells. It is most desirable to detect neoplasia in the pre-malignant stage, when the disease is not life threatening. Examples of such conditions include cervical squamous intraepithelial lesions; non-invasive bladder carcinoma; colorectal adenomas and dysplasia/intraepithelial neoplasia of the mucosa of the lung and upper aero-digestive tract.

Pre-malignant disorders are biologically different to the malignant conditions examined by Werness/Todorov *et al.* It was not demonstrated in, or obvious from, Werness/Todorov *et al* that MCMs would be expressed in abundance in the surface

layers of pre-malignant disorders and could therefore serve as biomarkers for the detection of such disease.

Werness/Todorov *et al* present data from only histological studies and do not refer to cytological samples. There is no suggestion that these papers refer to anything other than a potential role for MCMs in the histological analysis of malignancy. Todorov *et al* comment that MCM2 provides different information to PCNA and Ki67 and may therefore "prove to be an independently valuable aid in the diagnosis and prognosis of human tumours". It is clear from the data in Todorov *et al* that this statement refers to tissue sections. Werness refers only to use of tissue in Wetern blot and tissue sections.

There is no reference in the cited art to cytological diagnosis and no data is presented to suggest that MCMs could enable detection of cells taken or released from the surface of an epithelial surface that can then be used diagnostically as in the presently claimed invention.

Indeed, the Western blotting experiments were carried out on solubilized protein lysates, with no remaining cellular or tissue structure.

In summary, the present inventors made several original and non-predictable observations that led them to conclude that MCMs would serve as biomarkers for screening for neoplasia by analysis of surface cells (as opposed to histological analysis of intact tissues). In particular, they observed that MCMs had the following properties in neoplasia:

Abundance (giving detection sensitivity);

Expression in appropriate cellular compartments (i.e. surface cells);

Abundance at the surface of pre-malignant as well as malignant conditions.

As the specification indicates, clear differences in pattern and amount of expression of MCMs are indicative of the presence of dysplastic or neoplastic cells. Illustrating with reference to cervical tissue, there is high-level staining of abnormal cells, and full-thickness staining in LSIL and HSIL samples. This contrasts with only basal and parabasal layer staining of normal tissues. This allows for determination of the presence of dysplastic or neoplastic cells by determination of the pattern of anti-MCM antibody binding. Furthermore, "positional information" - an expected difference in MCM distribution for a sample containing dysplastic or neoplastic cells compared with normal - can be taken into account by means of the method of sampling, as is reflected in the pending claims. Thus, for example, with the cervix a smear can be taken from the epithelial surface where in a normal tissue no anti-MCM2 binding is seen. The presence of binding in a cervical smear is indicative of the presence of dysplastic or neoplastic cells.

The means of sampling can be chosen so that only a region is sampled in which no or little binding is seen if the tissue is normal - in which case the presence of any binding or a change in binding is indicative of the presence of dysplastic or neoplastic cells, as is the case with a cervical smear. Other sample types that take into account positional information as taught in the specification include urine and stool samples, along with others, as recited in the claims, wherein cells originate from epithelial surfaces.

None of the properties identified above was literally or inherently disclosed in either Werness or Todorov *et al.* or obvious from Todorov and Murphy (Clinical Oncology, American Cancer Society, 2nd edition, 1995, pages 553-554). There was

therefore no prediction from either paper that the method for detecting neoplasia in clinical samples would be successful.

The applicants respectfully submitted that it is one thing to take known tumor samples in tissue section or solubilized extract and known normal samples and to show a difference in staining for a target antigen as between the tumor and normal, as in Werness/Todorov, but quite a different thing to discover and demonstrate that there is an abnormal abundance and pattern of target expression that allows for clinical sampling and testing of material from individuals for whom it is not known whether or not they have any cellular growth abnormality. In performing a clinical or diagnostic test on an individual and especially when screening a population of individuals, such as is done on a massive scale for example for cervical cellular growth abnormality, it is important to be able to take a sample from the individual easily and quickly and to have a test that is accurate, sensitive and subject to rapid through-put.

The presently claimed invention meets these criteria, but it was not taught in the cited art, nor obvious therefrom that a clinical standard of diagnosis with a potential for rapid through-put could be achieved by targeting MCM2. The Werness and Todorov disclosures are limited to histological analysis of known tumor or normal tissue sections, are further limited to analysis with no use of positional information, and provide no indication that the claimed subject-matter could or should be possible.

The Materials and Methods section of Todorov et al. describes preparation of the samples tested. For immunohistochemistry, tissue sections of known tumor and normal samples were obtained. For Western blotting, the tissue and tumor samples were solubilized and boiled – this leaving a lysate containing protein that was run on a gel

and then transferred to a membrane on which target protein was bound with antibody. Intensity of reaction on the immunoblots was determined. Such an approach produces only crude information on abundance of protein present, and nothing about where the components of the protein preparation came from in the tissue studied. Todorov et al. indicate no interest in positional information within the tissues studied, save for noting of MCM2 localization to chromatin in nuclei of cells in G1 of the cell cycle. The Werness et al. abstract reports similar results to the Todorov paper. Again, tissue sections and Western blots of known tumor and normal tissue are all that were studied in the cited art.

The Examiner suggests "since the tissues tested were from a multitude of primary human tumors and normal tissues, the art reads on the screening of a population of individuals". See, page 5 of the Office Action dated February 25, 2003 (Paper No. 11).

Distinction from the art has already been explained above. However, it is further pointed out that "screening a population of individuals", as set out in claims 105, 109, 110 and 111, is distinct from the kind of experiments performed in the cited prior art.

Screening of a population involves taking samples from different members of the population to investigate whether in the case of each member there is evidence of abnormality. A common example practiced on a massive scale is screening for cervical abnormality. Other examples are colon and prostate. In screening a population it is not known in advance whether any of the individuals of the population have any abnormality, or, if any do, which do. The point of "screening a population" is to look for evidence in order to identify individuals, if any, with a health risk or disease state. This

is wholly different from testing of known tumor samples and known normal samples for antibody binding to target as in the cited prior art. Screening of a population is looking for rare events that will not occur in most samples – i.e. evidence of abnormality – without any prior knowledge of in which samples – indeed if in any samples at all – the event occurs.

Withdrawal of the Section 102 rejections are requested.

The Section 103 rejection of claims 88-100 and 105-109 over Todorov in view of Murphy is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

The Examiner cites Todorov et al. in view of Murphy et al., concluding that "It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to include the testing of cervical neoplasia and or a test cervical smear in the method as set forth by Todorov et al ...". See, page 1 of Paper No. 11.

Todorov has been discussed and distinguished above. Todorov does not disclose a method of determining the presence or absence of dysplastic or neoplastic cells in a test sample, since the samples were known already to contain tumor or non-tumor material. Moreover, Todorov does not contain any disclosure that would have suggested employing a test sample that comprises a specimen selected from the group consisting of sputum, bronchio-alveolar lavage specimens, urine, brushings from the alimentary tract, and cervical, fecal or urine cytology smears. Todorov does not provide any indication that an abnormal pattern of MCM expression at epithelial surfaces was or could or should be employed in a method as presently claimed, on samples as set out, with clinically relevant diagnostic accuracy.

Murphy does not remedy the deficiencies of Todorov compared with the presently claimed invention. Specifically, Murphy contains no disclosure related to any MCM protein whatsoever and as such cannot render obvious the presently claimed invention, whether applied to the cervix or any other sample as defined in the present claims. Furthermore, in applying Murphy with Todorov, the Examiner primarily refers to Todorov, only employing Murphy in relation to teaching about adenocarcinoma of the cervix. In relation to what the Examiner calls "certain gynecological tumor samples" (id.) the Examiner refers to Todorov as support for the contention of obviousness, despite the fact that Todorov does not contain any disclosure relating to the cervix and no indication that MCM2 protein is expressed in a differential manner at the surface in abnormal cervix (or any other tissue) compared with normal. For cervical smear sampling it is required, as reflected in the present invention, that there is abnormal MCM expression at the surface.

The present application contains experimental evidence demonstrating that MCM protein is present in abnormal cervix at the surface in a way that allows sampling via smears to be an effective means of providing material for analysis, including in a screening program. The application also shows that markers Ki67 and PCNA are not expressed with the same pattern, and thus that they are not useful in this way. See for example page 40, line 26 to page 41 line 9, and page 41, line 21 to page 42, line 6, page 47, line 25 to page 48, line 7, and so on. However, Todorov reports (page 74, column 2) only that MCM2 staining variations were greater in degree than for PCNA, and that (page 75, column 2) in normal lobules staining was negative for MCM despite

some staining for PCNA and Ki-67. The findings underlying the present invention are not obvious from Todorov, Murphy or a combination thereof.

Withdrawal of the Section 103 rejection is requested.

The applicants note the Examiner's recognition that unamended claims 101-104 are patentable over the art. As discussed, the inventors' contribution to the art is broader than these claims: it was not literally or inherently disclosed in the cited art, or obvious therefrom, to employ samples as defined in the pending claims in a method as defined; it was not literally or inherently disclosed in the cited art, or obvious therefrom that MCM protein expression could be used for clinical diagnosis of dysplasia or neoplasia by sampling of cells from epithelial surfaces, whether taken from the surfaces by means of a swab or brush, for example, or sloughed or exfoliated into bodily fluid, such as urine.

Reconsideration and withdrawal of the rejections are respectfully requested.

Return of an initialed copy of the attached PTO-1449 Form, pursuant to MPEP §609, is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested.

The Examiner is requested to contact the undersigned if anything further is required in this regard.

Respectfully submitted,

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